

CLAIM OR CLAIMS

I/WE CLAIM:

1. A method of performing targeted modifications of human embryonic stem (ES) cells, the method comprising the steps of
obtaining copies of a genetic construct which includes a foreign gene and which has regions at either of its ends homologous with a pair of selected regions in the genome of the ES cells so that homologous recombination will occur between the genetic construct and the selected regions of the genome of the stem cells;
electroporating the copies of the genetic construct into stem cell in culture; and
identifying cells which contain the genetic construct.
2. A method as claimed in claim 1 where the genetic construct include a marker gene.
3. A method as claimed in claim 2 wherein there is no promoter on the marker gene in the genetic construct, the genetic construct being inserted into the ES cells in a location in the genome of the ES cells wherein the marker gene is expressed only in cells in a desired state of differentiation.
4. A method as claimed in claim 2 wherein there is a tissue specific promoter driving the expression of the marker gene in the genetic construct, the tissue specific promoter being active only in cells in a desired state of differentiation.
5. Human cells in culture derived from human embryonic stem cells, the cells comprising in their genome an inserted genetic construct which knocks out the functioning of a gene which would otherwise be expressed in those human cells in culture.
6. Human cells in culture derived from human embryonic stem cells, the cells comprising in their genome an inserted genetic construct which introduced a mutation into a native gene in those human cells in culture.

7. A method of purifying cells of a defined lineage from cultures of human embryonic stem (ES) cells, the method comprising the steps of

obtaining copies of a genetic construct which has regions at either of its ends homologous with a pair of selected regions in the genome of the ES cells so that homologous recombination will occur between the genetic construct and the selected regions of the genome of the stem cells, the genetic construct including a marker gene which will be expressed only in cells of the defined lineage;

electroporating the copies of the genetic construct into stem cell in culture; and

identifying cells which express the marker gene in the genetic construct and purifying those cells from other cells.

8. A method as claimed in claim 7 wherein the marker gene includes a promoter which is active to express a gene only in cells of the desired lineage.

9. A method as claimed in claim 7 wherein after the electroporating step, the ES cells are permitted to differentiate.

10. A method as claimed in claim 7 wherein the marker gene expresses a fluorescent gene product and the identifying and purifying is performed by fluorescence activated cell sorting.

11. A culture of differentiated human cells derived from human ES cells and purified by the method of claim 7 for cells of a desired lineage.

12. A method for purifying cells of a defined lineage derived from human embryonic stem (ES) cells, the method comprising the steps of

purifying cells of the defined lineage by the method of claim 6;

analyzing the gene expression pattern of the purified cells to identify genes expressed in the cells of the defined lineage which are characteristic of that lineage;

culturing non-transformed ES cells so that the ES cells initiate differentiation; and

purifying the cells of the defined lineage based upon the expression of the genes identified in the analyzing step.

13. A method as claimed in claim 12 wherein the defined lineage is undifferentiated cells wherein the genes identified include genes for the cellular factors CD124, CD113, FGF-R, c-Kit, and BMP-4, and wherein the purification step is performed by testing cells for expression of at least one gene selected from the groups consisting of CD124, CD113, FGF-R, c-Kit, and BMP-4.

14. Human cells in culture derived from human embryonic stem cells, the cells comprising in their genome an inserted genetic construct which expresses an inserted gene only when the human cells are in a desired state of differentiation.

15. Human cells in culture as claimed in claim 14 wherein the desired state of differentiation is an undifferentiated state.

16. Human cells in culture as claimed in claim 14 where the gene is a marker gene the expression of which can be observed visually.